43. The mixture of claim 35, wherein the target RNA is contained within a nucleic acid sequence which encodes a protein associated with HIV or HCMV.

44. The kit of claim 36, wherein the target nucleic acid is contained within a nucleic acid sequence which encodes a protein associated with HIV or HCMV.

45. The plasmid of claim 37, wherein the target nucleic acid is contained within a nucleic acid sequence which encodes a protein associated with HIV or HCMV.

II. Remarks

The present application has been remanded to the Examiner by the Board of Patent Appeals and Interferences (the "Board") for action consistent with the Board's decision mailed August 8, 1996. In its decision, the Board noted, inter alia, that the Examiner's Answer contained a new ground of rejection. In view of this new ground of rejection, Applicants submit the amendments set forth above to further prosecution of this application. As will be set forth below, these amendments also necessitate that the Examiner declare an interference between this application and U.S. patent no. 5,476,774.

A. Identification Of The Patent Which Includes Subject Matter Which Interferes With The Application

The patent which claims subject matter which interferes with subject matter claimed in the present application is U.S. patent no 5,476,774 (the "'774 patent") issued on December 19, 1995 to Wang et al. For "QUANTITATION OF NUCLEIC ACIDS USING THE POLYMERASE CHAIN REACTION." The '774 patent was issued from Ser. No. 028,464, filed March 9, 1993, which purports on its face to be a continuation of Ser. No. 413,623, filed September 28, 1989 (now U.S. patent no. 5,219,727), which is a continuation-in-part of Ser. No.

396,986, filed August 21, 1989, abandoned. Hoffman-La Roche Inc. is the assignee on the face of the patent.

B. Presentation Of A Proposed Count

The following count is proposed for purposes of interference.

1. An amplification reaction mixture for the quantitation of a target nucleic acid segment in a biological sample, said reaction mixture comprising:

said target nucleic acid;

a predetermined initial amount of an internal standard nucleic acid segment for quantitation of a target nucleic acid, wherein said internal standard binds the same primers as are bound by said target nucleic acid segment; and

an oligonucleotide primer pair wherein said primer pair can serve to amplify said internal standard and said target nucleic acid, wherein following amplification said standard and target amplified nucleic acid segments are distinguishable by size or by use of internal hybridization probes.

The Proposed Count corresponds exactly to patent claim 15, which is the broadest claim in the patent. It is believed that the Proposed Count encompasses all of the subject matter of the remaining patent claims.

C. Identification Of Claims Of The '774 Patent Corresponding To The Count

As noted in 37 C.F.R. 1.606, all claims that "define the same patentable invention as a count shall be designated to correspond to the count" and "any single patent claim designated to correspond to the count will be presumed...not to contain separate patentable inventions."

Claim 15 is the broadest claim in the '774 patent. This claim is directed to an amplification reaction mixture. The other three independent claims in the '774 patent, claims 1, 5 and 17, are directed to subject matter which do not appear to constitute separate patentable inventions. The remaining claims all depend from claims 1,5,15 or 17. Applying the provisions of 1.606 to these claims, claims 1-18 in the '774 patent are presumed to be directed to the same patentable invention. Accordingly, claims 1-18 should be designated as corresponding to the Proposed Count.

Applicants claims 34-45 should also be designated as corresponding to the Proposed Count. These claims define the same patentable invention as the patent claims. Claims 34, 35, 36 and 37 correspond to claims 15, 17, 5 and 1 in the '774 patent, respectively. To assist the Examiner in this regard, Applicants attach Appendix A which is a chart providing an element-by-element recitation of the Proposed Count (i.e., claim 15 in the '774 patent which corresponds to claim 34 of this application -- the only difference between claim 15 in the '774 patent and claim 34 in this application is that claim 34 refers to a "control sequence," whereas claim 15 refers to an "internal standard nucleic acid segment" -- these are two ways of saying the same thing) and an indication of the passages in the application where, at the very least, the Proposed Count finds support in the present application. Also in Appendix A, Applicants set forth where support for claims 35-45 can be found in the present application. With respect to claim 37, the omission

from claim 37 of language concerning "upstream" and "downstream" primer hybridization sites is of no consequence in determining whether or not claim 37 and claim 1 of the '774 patent are directed to the same patentable invention. The omitted language simply refers to inherent structural features of nucleic acids necessary for PCR to occur.

The designation of Applicant's claims as corresponding to the single Proposed Count is not to be construed as Applicant's acquiescence in the correctness of the designation or the correctness of the count, or a concession that the claims are directed to the same patentable invention. Applicants reserve the right to challenge the propriety of the Proposed count, the designation of any claim as corresponding the Proposed count, and the patentability of any claim during the preliminary motion period in an interference, or otherwise.

D. 35 U.S.C. 135(b) Is Satisfied

The '774 patent issued on December 19,1995 which is less than one year prior to the filing of the present claims/request. Therefore, the provisions of 35 U.S.C. 135 (b) have been satisfied.

III. Applicants Are Entitled To An Earlier Filing Date Than Wang et al . Under 35 U.S.C. 120

Applicants request that they be accorded the benefit of the filing date of at least Ser. No. 07/148,959, filed January 27, 1988 (the "'959 application"). A copy of the '959 application is attached hereto as Appendix B. The present application is a continuation-in-part application claiming priority from, inter alia, the '959 application. The '959 application contains an enabling

disclosure of the subject matter corresponding to the Proposed Count. See, e.g., page 3, line 23 through page 5, line 11.

IV. Conclusion

Applicants respectfully request that an interference be declared employing the Proposed Count set forth above with claims 1-18 of the '774 patent and claims 34-45 of the present application, all of which it is believed should be designated as corresponding to the Proposed Count.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned attorney at (202) 783-6040.

Respectfully submitted,

By _____

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APPENDIX A

1. **Proposed Count/'774 Claim 15**	**Applicant's Disclosure**
An amplification reaction mixture of a target nucleic acid segment in a biological sample, said reaction mixture comprising:	For identification and quantification purposes it is preferred to amplify the viral RNA sample, typically from virus infected T-4 lymphocytes present in peripheral blood, simultaneously with at least one other RNA sequence to provide a positive control and reduce the risk of false negative data. A plurality of first and second primer pairs is provided, one such pair for each RNA sequence to be amplified. The amplification procedure is otherwise accomplished as previously described. (Page 4, lines 1-8.) Here, the target nucleic acid segment is viral RNA and the biological sample is blood.
said target nucleic acid	See disclosure above.

1. **Proposed Count/'774 Claim 15**	**Applicant's Disclosure**
a predetermined initial amount of an internal standard nucleic acid segment for quantitation of a target nucleic acid,	For identification and quantification purposes it is preferred to amplify the viral RNA sample, typically from virus infected T-4 lymphocytes present in peripheral blood, simultaneously with at least one other RNA sequence to provide a positive control and reduce the risk of false negative data. A plurality of first and second primer pairs is provided, one such pair for each RNA sequence to be amplified. The amplification procedure is otherwise accomplished as previously described. (Page 4, lines 1-8.) An additional aid to quantitation of virus levels in patient samples is provided by a reference RNA which can be amplified and detected by the same oligonucleotide used for authentic virus RNA samples. Such a reference RNA may be a "minigene" or a "maxigene" formed by a multi-base pair insert into or deletion of at least about 20 nucleotides from a unique site. (Page 6, lines 15-21.) Such "minigenes" and "maxigenes" not only provide an internal control but also an additional aid to quantitation. Because the quantity of "maxigene" minigene RNA originally included in the amplification reaction is known, the amount of signal obtained from the maxi or minigene amplification product can be related to the signal obtained from the patient sample. Hence, the relative quantitation of the original amount of authentic HIV-1 in the patient sample is provided. (Page 7, lines 1-8.) Here, the internal standard nucleic acid segment is a "control sequence" "maxigene" or "minigene." The quantity of this element in the reaction mixture is known (i.e., predetermined).
wherein the internal standard binds the same primers as are bound by said target nucleic acid segment; and	An additional aid to quantitation of virus levels in patient samples is provided by a reference RNA which can be amplified and detected by the same oligonucleotide used for authentic virus RNA samples. Such a reference RNA may be a "minigene" or a "maxigene" formed by a multi-base pair insert into or deletion of at least about 20 nucleotides from a unique site. (Page 6, lines 15-21.)

1. **Proposed Count/'774 Claim 15**	**Applicant's Disclosure**
an oligonucleotide primer pair wherein said primer pair can serve to amplify said internal standard and said target nucleic acid,	In general the method of the invention entails utilizing a sample RNA which has or may have a target viral sequence as a template for amplification by PCR. A first oligonucleotide primer for the target viral sequence is annealed to the template for extension through the target sequence to produce a first extension product having an RNA template strand and a DNA primer extension strand. The first extension product is denatured and the separated RNA template and DNA primer extension strands are annealed, respectively to the first primer and to a second primer complementary to the DNA primer extension strand. The first and second primers are positioned for extension through the target sequence on the template and its complement on the primer extension strand. The first and second primers are extended to produce a second primer extension product which is denatured, the first and second primers are again annealed to the separated template and primer extension strands, and again extended and the resulting extension products denatured. The process is repeated for the number of cycles deemed appropriate to achieve the desired degree of amplification. (Page 1, lines 24 - page 2, line 11.) An additional aid to quantitation of virus levels in patient samples is provided by a reference RNA which can be amplified and detected by the same oligonucleotide used for authentic virus RNA samples. Such a reference RNA may be a "minigene" or a "maxigene" formed by a multi-base pair insert into or deletion of at least about 20 nucleotides from a unique site. (Page 6, lines 15-21.)
wherein following amplification said standard and target amplified nucleic acid segments are distinguishable by size or by use of internal hybridization probes.	The transcription product of this clone [i.e., a "maxigene" or internal standard] is 21 bases longer than the authentic HIV-sequence but still hybridizes with the 25-mer probe HIVC. It is therefore distinguishable by size from the authentic viral product. (Page 6, lines 26-29.)

2. **Claim 35**	**Applicant's Disclosure**
35. A reverse transcription reaction mixture for reverse transcribing a target mRNA suspected of being present in a biological sample, said reaction mixture comprising a predetermined initial amount of a control sequence cRNA, a target RNA, and a target-specific primer for initiating cDNA synthesis, wherein said primer can serve to initiate reverse transcription of a nucleic acid segment contained within said control sequence cRNA together with a segment contained within the particular target nucleic acid, and wherein said control sequence is further distinguished by having a hybridization site identical in sequence to a hybridization site in said target nucleic acid, whereby following reverse transcription the resulting target and control sequence cDNAs can serve as templates for amplification for providing control sequence and target amplified nucleic acid segments which are distinguishable by size.	See disclosures above, which are directed in part to quantitation of viral RNA.

3. **Claim 36** 36. A kit for the quantitation of a target nucleic acid segment in a biological sample comprising individual containers which provide: A predetermined initial amount of a control sequence for quantitation of a target nucleic acid segment; and An oligonucleotide primer pair can serve to amplify said control sequence and said target nucleic acid.	See disclosures above. Kits contemplated by the invention include self-contained appropriate quantities of primers and probes for use in the practice of the invention. A typical kit for the detection and quantification of HIV-1 virus in a patient peripheral blood sample includes vials or similar separate containers filled with, for example, 20 picomoles/microliters (in sterile H_2O) each of HIVA, HIVB or HIVC. A reference RNA (~10,000 copies/microliter) is prepared in sterile DEPC treated water. Such kits include reagents and instructions necessary to conduct the appropriate amplification and hybridization procedures. (Page 13, lines 1-11.)
4. **Claim 37** 37. A plasmid for use as an internal control for quantitation of a target nucleic acid sequence contained within a sample which plasmid comprises: A control sequence comprising two sequences which provides primer hybridization sites within said target nucleic acid sequence such that a primer pair will function in a PCR reaction to amplify said control sequence and said target nucleic acid segment, wherein upon amplification said control sequence and said target segments can be distinguished by size.	See disclosures above. Similar procedures can be used as a quantitative assay of HCMV sequences. A segment of the cDNA derived from the major IE gene IE1 is subcloned into the transcription vector pTZ18U (BioRad), and includes nucleotides 1185-1331. A small insertion accomplished either by cloning or by site directed mutagenesis is made in this segment whch permits distinction between the PCR-amplified viral RNA and cellular amplified transcripts. By including a fixed amount of this plasmid HCMV RNA or DNA in every sample to be amplified, it is possible to measure the amount of viral DNA or RNA using the in vitro sample as an internal standard. (Page 7, lines 9-19)
38. The mixture of claim 34, wherein the control sequence is a maxigene.	See disclosures above.
39. The mixture of claim 35, wherein the control sequence is a maxigene.	See disclosures above.

40. The kit of claim 36, wherein the control sequence is a maxigene.	**Applicant's Disclosure** See disclosures above.
41. The plasmid of claim 37, wherein the control sequence is a maxigene.	See disclosures above.
42. The mixture of claim 34, wherein the target nucleic acid is contained within a nucleic acid sequence which encodes a protein associated with HIV or HCMV.	See disclosures above.
43. The mixture of claim 35, wherein the target RNA is contained within a nucleic acid sequence which encodes a protein associated with HIV or HCMV.	See disclosures above.
44. The kit of claim 36, wherein the target nucleic acid is contained within a nucleic acid sequence which encodes a protein associated with HIV or HCMV.	See disclosures above.
45. The plasmid of claim 37, wherein the target nucleic acid is contained within a nucleic acid sequence which encodes a protein associated with HIV or HCMV.	See disclosures above.